EXHIBIT 1

THE TALENT ARTICLE:

"PILOT STUDY OF ORAL POLYMERIC N-ACETYL-D-GLUCOSAMINE AS A POTENTIAL TREATMENT FOR PATIENTS WITH OSTEOARTHRITIS"

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Pilot Study of Oral Polymeric *N*-acetyl-D-glucosamine as a Potential Treatment for Patients with Osteoarthritis

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ABSTRACT

Glucosamine and its derivatives, such as glucosamine sulfate and *N*-acetyl-D-glucosamine (NAG), have been shown to be effective in the treatment of patients with osteoarthritis. Unfortunately, the half-life of glucosamine in the blood is relatively short; therefore, a sustained-release form of the compound would be highly desirable. The purpose of this pilot study was to determine whether the polymeric form of NAG (POLY-Nag®) could provide a longer-lasting oral source of NAG. Ten healthy subjects each ingested 1 g/d of either NAG or POLY-Nag for 3 days. After a 4-day washout period, each subject was crossed over to receive the other compound for 3 days. Serum samples were collected and analyzed using high-performance liquid chromatography. Results show that orally ingested NAG and POLY-Nag are absorbed, resulting in increased serum levels of NAG, and POLY-Nag appears to be at least as effective as NAG. Serum levels of NAG had decreased by 48 hours after cessation of ingestion of NAG or POLY-Nag but were still above baseline levels. Increases in serum glucosamine levels indicate that NAG and POLY-Nag are converted to glucosamine in vivo. In conclusion, POLY-Nag may provide a source of serum glucosamine for treatment of patients with osteoarthritis. Longer and more rigorous pharmacokinetic and clinical studies need to be done.

INTRODUCTION

The incidence of osteoarthritis increases with age, and it is found in most persons older than 65 years.[1] The importance of osteoarthritis is growing as the world's population ages. Although the pain associated with this disease can be managed with a variety of nonsteroidal anti-inflammatory drugs (NSAIDs), this treatment does not address the underlying degenerative disorder.

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Glucosamine and *N*-acetyl-D-glucosamine (NAG) are constituents of glycoproteins, proteoglycans, glycosaminoglycans (GAGs), and other building blocks of connective tissue. Glucosamine is the substrate for GAG biosynthesis and also stimulates its synthesis, inhibits its degradation, and appears to be directly involved in the repair of damaged cartilage.[2] These natural products may represent a more desirable method of treating osteoarthritis than NSAIDs, because they directly alter cartilage metabolism and are nontoxic, with virtually no side effects.[2–5] Efficacy and safety of these agents have been reported in randomized, placebo-controlled, double-masked studies.[6–9]

Uniformly radiolabeled glucosamine has exhibited good absolute bioavailability after oral administration, along with a trophism for articular cartilage.[10,11] However, the half-life of glucosamine in the blood is relatively short.[5,8,9] Thus a longer-lasting or sustained-release form of the compound would be highly desirable.

NAG has been shown to be hydrolyzed to glucosamine both in vitro and in vivo.[12,13] The polymeric form of NAG (chitin, or poly [*N*-acetylglucosamine] [POLY-Nag®; Lescarden, Inc., New York, New York]) is nontoxic and inexpensive and could potentially provide a longer-lasting source of NAG. It was not known whether this polymer could provide an oral source of NAG.

The purpose of this pilot study was to determine whether POLY-Nag could serve as an oral source of NAG by examining the appearance and disappearance of NAG and glucosamine in serum from subjects who orally ingested either NAG or POLY-Nag. The intent of the study was not to gain statistical pharmacokinetic information, but rather to establish whether POLY-Nag is absorbed and degraded and thus provides a source of serum NAG. In addition, we wanted to define preliminary information on the half-life of POLY-Nag such that a larger, more definitive study could be designed to explore the use of this agent.

SUBJECTS AND METHODS

All test materials were supplied by Lescarden, Inc., and were verified to meet the requirements of the United States Pharmacopeia Microbial Limits Test.[14]

This randomized, crossover study included 10 healthy subjects (5 men and 5 women; age range, 36 to 50 years) who were randomly divided into groups, designated as A and B. Subjects were obtained as volunteers from the students and employees of the University of North Texas Health Science Center. Names of all volunteers were written on slips of paper and placed according to gender in one of two nontransparent canisters. Groups were randomized by alternately drawing from each canister. The study was carried out according to the principles of the Declaration of Helsinki. The University of North Texas Health Science Center Institutional Review Board (Fort Worth, Texas) reviewed and approved the study, and informed consent was obtained from all subjects.

On day 1 of the study, a fasting blood sample was collected from each patient between 8 AM and 9 AM. Subjects in group A then orally ingested 1 g of NAG, and subjects in group B ingested 1 g of POLY-Nag. On day 2 the subjects again ingested 1 g of the appropriate test substance before breakfast. On day 3 a fasting blood sample was obtained at time zero (T0) (between 8 AM and 9 AM) to coincide with the time at which the subject had donated a

fasting blood sample for the pretest. No blood was drawn on day 2 of ingestion. The subjects then consumed the final 1 g of the appropriate test substance. Nonfasting blood samples were obtained at 1, 2, 4, and 8 hours after ingestion. Fasting blood samples were obtained at 24 and 48 hours thereafter. Days 4 through 7 were a washout period during which subjects did not consume any of the test substance but continued their normal diets. On day 8, a fasting blood sample was obtained from all subjects again at 8 AM to 9 AM, and the regimen for the groups was switched; group A ingested POLY-Nag, group B ingested NAG, and the procedure was repeated as previously described.

Blood samples were analyzed with a high-performance liquid chromatography procedure based on published methods for amino sugar analysis.[15] The chromatographic system consisted of a Hewlett-Packard 1090M liquid chromatograph/workstation equipped with a diode array detector (Hewlett-Packard, Palo Alto, California). Separation was by isocratic elution at 40 °C from a 300 × 7.8-mm Rezex™ organic acid column (minimum efficiency >7500 pm) (Phenomenex, Torrance, California) with 0.005 N H_2SO_4 at a flow rate of 0.6 mL/min. The analytical conditions had been developed for resolution of the amino sugar/acetylated amino sugar components of physiologic fluids, and the diode array detector was optimized at 193 nm for maximum sensitivity and minimum interference. NAG and glucosamine were identified and quantified by injection of the standards as independent aqueous solutions as complements of standard mixtures, and as "spiked" additions to serum samples. The limit of quantitation of the method was 0.5 nmoL (0.11 μg) for NAG and 10 nmoL (1.8 μg) for glucosamine. The system was calibrated with standards before, during, and after the study to ensure that no significant changes occurred due to wear and tear or aging of the system. The chromatographic data were transferred to a spreadsheet and statistically tested with analysis of variance (single factor, two-factor without replication, and two-factor with replication) and paired *t* tests.

RESULTS

The table summarizes the concentrations of NAG in serum after ingestion of 1 g/d of either NAG or POLY-Nag at all time points. Because of individual differences and because subjects were not fasting for all time periods, it is more informative to view the quantitative responses of each individual. For example, comparison of serum NAG levels at T0 with pretest levels showed that 13 (65%) of the 20 samples taken from the 10 subjects demonstrated increased serum NAG concentrations. A similar number of NAG ingestors and POLY-Nag ingestors showed increased serum NAG concentrations (6 of 10 and 7 of 10, respectively). In addition, because the T0 sample was taken in the morning before subjects received the final dose of the drug, another important comparison is to contrast the pretest NAG level with the NAG level at 1 hour. In this case, 14 (70%) of the 20 samples demonstrated increases in serum NAG levels (8 of 10 NAG ingestors and 6 of 10 POLY-Nag ingestors). These data indicate that ingestion of either NAG or POLY-Nag results in absorption and a resulting elevation in serum NAG levels.

On cessation of ingestion of NAG or POLY-Nag, serum NAG levels did not immediately return to baseline levels (figure). At 24 hours after cessation of inges-

Table. Serum *N*-acetyl-D-glucosamine (NAG) levels (mean ± SD) in ingestors of NAG and polymeric NAG.

Time of Sample	Fasting	Serum NAG Levels (nmol/mL)	
		NAG Ingestors	POLY-Nag Ingestors
Pretest	Yes	10.3 ± 4.4	13.4 ± 7.0
Immediately before ingestion	Yes	15.4 ± 8.7	23.0 ± 18.3
Time after ingestion			
1 h	No	18.2 ± 10.0	15.6 ± 6.0
2 h	No	21.0 ± 18.4	24.3 ± 19.9
4 h	No	22.9 ± 16.7	23.7 ± 20.8
8 h	No	14.6 ± 9.3	13.8 ± 10.4
24 h	Yes	13.6 ± 12.0	18.6 ± 14.1
48 h	Yes	12.7 ± 8.3	23.0 ± 13.2

Note: 20 samples were collected in 10 patients.
Trademark: POLY-Nag® (Lescarden, Inc., New York, New York).

tion of either NAG or POLY-Nag, only 4 (20%) of the 20 samples showed decreased serum NAG levels. At 48 hours after cessation of ingestion, 55% of the samples still showed higher levels of serum NAG than at baseline, which suggests that clearance of NAG is not complete by 48 hours. Also, as seen in the figure, after completion of ingestion of the test substances, levels of serum NAG decreased in NAG ingestors but remained elevated in POLY-Nag ingestors.

Because glucosamine can arise from the hydrolysis (deacetylation) of NAG and is a normal breakdown product of NAG, serum glucosamine levels were also measured. Serum glucosamine levels were elevated over baseline values at 24 and 48 hours into the washout period. In fact, at 48 hours after final ingestion, serum glucosamine levels were elevated 10.2% and 12.8% over pretest levels for NAG ingestors and POLY-Nag ingestors, respectively. These data suggest that oral NAG and POLY-Nag are hydrolyzed to glucosamine before further metabolism or excretion. These data also corroborate the serum NAG data, suggesting that turnover is not complete by 48 hours, and in follow-up detailed pharmacokinetic evaluations, a longer washout period should be considered.

DISCUSSION AND CONCLUSIONS

Various drug therapies have been proposed for the treatment of patients with osteoarthritis. NSAIDs and analgesics address pain only and do not treat the underlying problem. Other so-called slow-acting drugs for osteoarthritis have been proposed to act by various mechanisms on synthesis, protection, and repair of cartilage; glucosamine sulfate is one of these drugs.[7] Mechanism studies in human chondrocytes have shown that glucosamine stimulates synthesis of GAGs and proteoglycans[16] and elicits mild anti-inflammatory activity by a mechanism

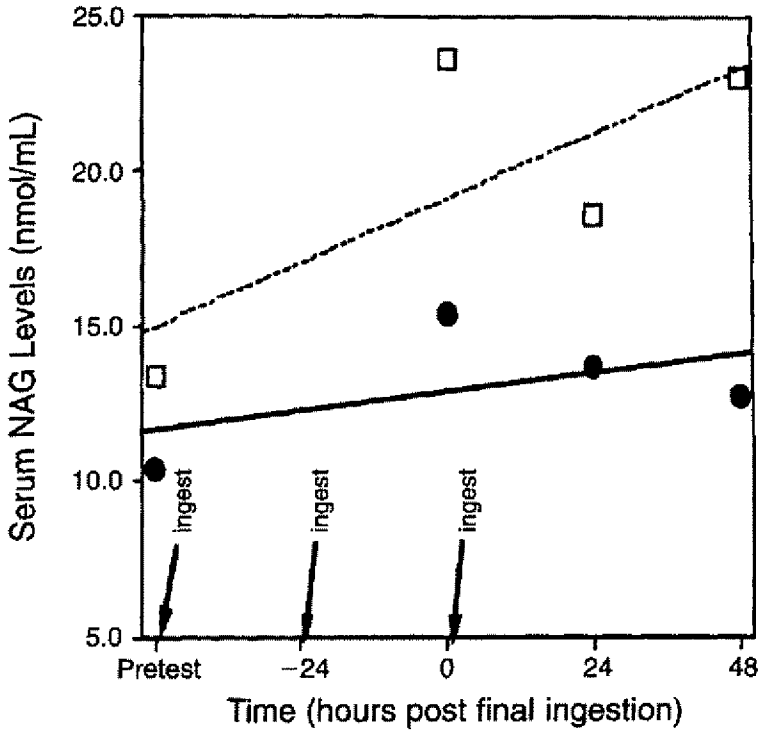

Figure. Effect of oral ingestion of *N*-acetyl-D-glucosamine (NAG) or polymeric NAG (POLY-Nag® [Lescarden, Inc., New York, New York]) on serum NAG levels. After fasting serum levels of NAG were determined (Pretest), 10 subjects ingested 1 g of either NAG (●) or POLY-Nag (□) once daily for 3 successive days (*arrows*). Immediately before ingestion on the third day (T = 0 hour) and at 24 and 48 hours after final ingestion, fasting blood samples were obtained and serum NAG levels were determined.

other than inhibition of prostaglandin synthesis.[17,18] In view of the efficacy and safety afforded by glucosamine, the use of this naturally occurring component of connective tissue in the treatment of patients with osteoarthritis is quite attractive. A major shortcoming has been the rapid clearance of glucosamine. A polymeric form of NAG that might provide a sustained-release form of glucosamine has much potential.

Chitin (POLY-Nag) is a normal component of shellfish such as crab, shrimp, and lobster. It is the second most plentiful natural polymer (after cellulose), and it is both biodegradable and nontoxic. For example, its oral median lethal dose in mice has been reported to be >16 g/kg body weight, which is similar to the median lethal dose of sucrose or sodium chloride.[19] Enzymatic degradation of POLY-Nag presumably occurs by one or more of several different hy-

drolyses, including chitinases, chitosanases, and lysozymes, which are widely distributed in many organisms, including mammals. The studies described herein suggest that POLY-Nag is indeed hydrolyzed to NAG and glucosamine in vivo.

All subjects maintained records detailing their dietary consumption and activities during the study. During this study the subjects reported no adverse effects. Compliance with the protocol was maintained without problem, and no differences in response were observed between men and women. Blood glucose levels did not show any significant variations attributable to either NAG or POLY-Nag ingestions. This finding is consistent with a previous study[5] that reported no increases in serum glucose levels after intravenous administration of NAG.

In conclusion, this study indicates that oral ingestion of 1 g/d of NAG or POLY-Nag increased the serum concentration of NAG. When the test substance was withdrawn, levels of serum NAG decreased and appeared to be at least partially hydrolyzed to glucosamine before further metabolism or excretion. POLY-Nag may provide a sustained level of serum glucosamine for treatment of patients with osteoarthritis, but longer and more rigorous pharmacokinetic and clinical studies should be undertaken.

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